AD-A033 796 ARMY MEDICAL RESEARCH INST OF INFECTIOUS DISEASES FR--ETC F/G 6/13
EFFECT OF HARVEST TIME OF RICKETTSIA RICKETTSII FROM DUCK EMBRY--ETC(U)
DEC 76 G L RUCH, D E HILMAS

UNCLASSIFIED

NL

| OF |
AD
A033796

END

DATE
FILMED
2-77

Effect of Harvest Time of *Rickettsia rickettsii* from Duck Embryo Cell Cultures on Rocky Mountain Spotted Fever Infection in the Rhesus Monkey

GERALD L. RUCH[1] AND DUANE E. HILMAS

United States Army Medical Research Institute of Infectious Diseases
Fort Detrick, Frederick, Maryland 21701

Running head: RMSF IN RHESUS MONKEYS

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

Address requests for reprints to: LTC Duane E. Hilmas, VC
USAMRIID, Fort Detrick
Frederick, MD 21701

[1]Present address: Laboratory Supply Co., Inc.
5006 Mooresville Rd.
Indianapolis, Indiana 46241

Approved for public release; distribution unlimited

15 Dec 76

Effect of Harvest Time of *Rickettsia rickettsii* from Duck Embryo Cell Cultures on Rocky Mountain Spotted Fever Infection in the Rhesus Monkey

GERALD L. RUCH[1] AND DUANE E. HILMAS

United States Army Medical Research Institute of Infectious Diseases

Fort Detrick, Frederick, Maryland 21701

Running head: RMSF IN RHESUS MONKEYS

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

Address requests for reprints to: LTC Duane E. Hilmas, VC
USAMRIID, Fort Detrick
Frederick, MD 21701

[1]Present address: Laboratory Supply Co., Inc.
5006 Mooresville Rd.
Indianapolis, Indiana 46241

ABSTRACT

This study was undertaken to determine the optimum harvest time for infected duck embryo cell (DEC) cultures to yield the highest rickettsial titers and greatest infectivity for rhesus monkeys. A consistently reproducible Rocky Mountain spotted fever-infected monkey model for pathogenesis studies and testing vaccine is produced when the inoculum given intravenously contains $\geq 10^{6.0}$ chick embryo median lethal dose organisms. This is optimally attained with day-4 DEC culture harvest material.

The rhesus monkey has served as a suitable host for the study of the pathogenesis and pathophysiology of Rocky Mountain spotted fever (RMSF) (4,6,7,9). However, to establish a reproducible RMSF infection in subhuman primates, it has been essential to grow stock inocula of Rickettsia rickettsii of high yield while maintaining their infectivity for the host. This report describes the optimum harvest time for rickettsiae-infected duck embryo cell (DEC) cultures, both in terms of titer and infectivity for rhesus monkeys.

The Sheila Smith strain of R. rickettsii was propagated in embryonated chicken eggs according to the methods of Stoenner et al. (8). Seed stock was stored at 70°C as a 50% yolk sac slurry in sucrose-phosphate-glutamate (SPG) buffer (1). Dilutions were made in SPG buffer, pH 7.2. The preparation of DEC cultures and the techniques for rickettsial growth and harvest have been described earlier (3). Rickettsiae were harvested from several inoculated flasks daily for six consecutive days after infection.

Infectivity of each harvest obtained on these six days was determined by titration of each sample by serial 10-fold dilutions in the yolk sacs of 6-day-old embryonated chicken eggs and by intraperitoneal (i.p.) inoculation of Hartley strain male guinea pigs (350-400 g). Guinea pig rectal temperatures were measured daily for 14 days, scoring any temperature greater than 40.0°C as positive indication of infection. Guinea pigs were also examined for other clinical signs of RMSF, such as scrotal swelling and erythema of footpads, ears, and scrotum. The chick embryo median lethal dose ($CELD_{50}$) and guinea pig IP median infectious dose ($GPIPID_{50}$) were calculated by the method of Reed and Muench (5).

Twelve rhesus monkeys of either sex, 3.2 to 4.5 kg weight, were allocated into groups and inoculated intravenously (i.v.) with 1 ml of harvest material. Two monkeys were inoculated with material from each day's harvest and grouped as follows: Group I received rickettsiae harvested on day 1 or 2 ($10^{4.5}$ $CELD_{50}$), group II received rickettsiae harvested on day 3 or 6 ($10^{5.5}$ $CELD_{50}$), and group III received organisms harvested on day 4 or 5 ($\geq 10^{6.0}$ $CELD_{50}$). Monkeys were monitored daily for 16 days to record incubation period (time from inoculation to onset of fever $\geq$ 40.0°C), duration of illness (onset of fever $\geq$ 40.0°C to death, or until temperature decreased to < 40.0°C in survivors), and time to death (time from inoculation to death). Pathological examinations were performed on all monkeys which died.

No difference in numbers of rickettsiae were apparent in stained (2) smears from the DEC cultures from days 3 to 6 postinfection, but peak titers of 6.2 and 7.5 logs occurred on day 4 by the respective $CELD_{50}$ and $GPIPID_{50}$ assay methods (Fig. 1). Rickettsial titers for day 3 were comparable to those for day 6 but were slightly less than those for days 4 and 5 (Fig. 1). RMSF infection was least severe, with 25% mortality, in monkeys which received $10^{4.5}$ $CELD_{50}$ of rickettsiae harvested from DEC cultures on day 1 or 2 (Table 1). Group II monkeys inoculated with $10^{5.5}$ $CELD_{50}$ of rickettsial organisms harvested from days 3 and 6 exhibited 50% mortality with a mean incubation period of 1.2 days (Table 1). The disease was 100% lethal only in those monkeys which received $\geq 10^{6.0}$ $CELD_{50}$ of rickettsiae from DEC culture material harvested on day 4 or 5 (Table 1). Duration of illness and time to death were generally shorter in this latter group (Table 1). All

monkeys that died exhibited lesions on histopathologic examination compatible with a diagnosis of RMSF.

In a subsequent experiment, a large number of DEC cultures were inoculated with R. rickettsii and harvested on day 4 postinoculation. The respective $GPIPID_{50}$ and $CELD_{50}$ titrations showed 7.5 and 6.3 logs of rickettsiae in this material, which is consistent with titers shown in Fig. 1. Twenty monkeys infected with this pool exhibited 100% morbidity and 90% mortality with nearly all deaths occurring between days 3 and 6 postinfection (4).

Clearly, the yield of rickettsial organisms depends upon the harvest time of inoculated DEC cultures. Stained smears do not distinguish between live and dead rickettsiae; therefore, they should not be used as a means of determining infectivity. Guinea pig and chicken embryo titrations indicate that materials harvested on day 4 or 5 yielded the highest titers and showed the greatest infectivity (shortest incubation period, highest mortality and earliest deaths) for rhesus monkeys. This is approximately 2 days earlier than the previously recommended harvest time for DEC culture material (3). Inocula from earlier (days 1 to 3) or later (day 6) harvest materials were not consistently infective for monkeys. With the exception of materials harvested on days 1 and 2, the guinea pig assay appears to be slightly more sensitive than the chicken embryo assay.

LITERATURE CITED

1. Bovarnick, M. R., J. C. Miller, and J. C. Snyder. 1950. The influence of certain salts, amino acids, sugars and proteins on the stability of rickettsiae. J. Bacteriol. 59:509-522.
2. Gimenez, D. F. 1964. Staining rickettsiae in yolk-sac cultures. Stain Technol. 39:135-140.
3. Kenyon, R. H., W. M. Acree, G. G. Wright, and F. W. Melchior, Jr. 1972. Preparation of vaccines for Rocky Mountain spotted fever from rickettsiae propagated in cell culture. J. Infect. Dis. 125:146-152.
4. Moe, J. B., G. L. Ruch, R. H. Kenyon, J. D. Burek, and J. L. Stookey. 1976. Pathology of experimental Rocky Mountain spotted fever in rhesus monkeys. Vet. Pathol. 13:69-77.
5. Reed, L. J., and H. Muench. 1938. A simple method of estimating fifty per cent endpoints. Am. J. Hyg. 27:493-497.
6. Sammons, L. S., R. H. Kenyon, G. T. Burger, C. E. Pedersen, Jr., and R. O. Spertzel. 1976. Changes in blood serum constituents and hematologic values in Macaca mulatta with Rocky Mountain spotted fever. Am. J. Vet. Res. 37:725-730.
7. Saslaw, S., H. N. Carlisle, G. L. Wolf, and C. R. Cole. 1966. Rocky Mountain spotted fever: clinical and laboratory observations of monkeys after respiratory exposure. J. Infect. Dis. 116: 243-255.
8. Stoenner, H. G., D. B. Lackman, and E. J. Bell. 1961. Factors affecting the growth of rickettsias of the spotted fever group in fertile hens' eggs. J. Infect. Dis. 110:121-128.

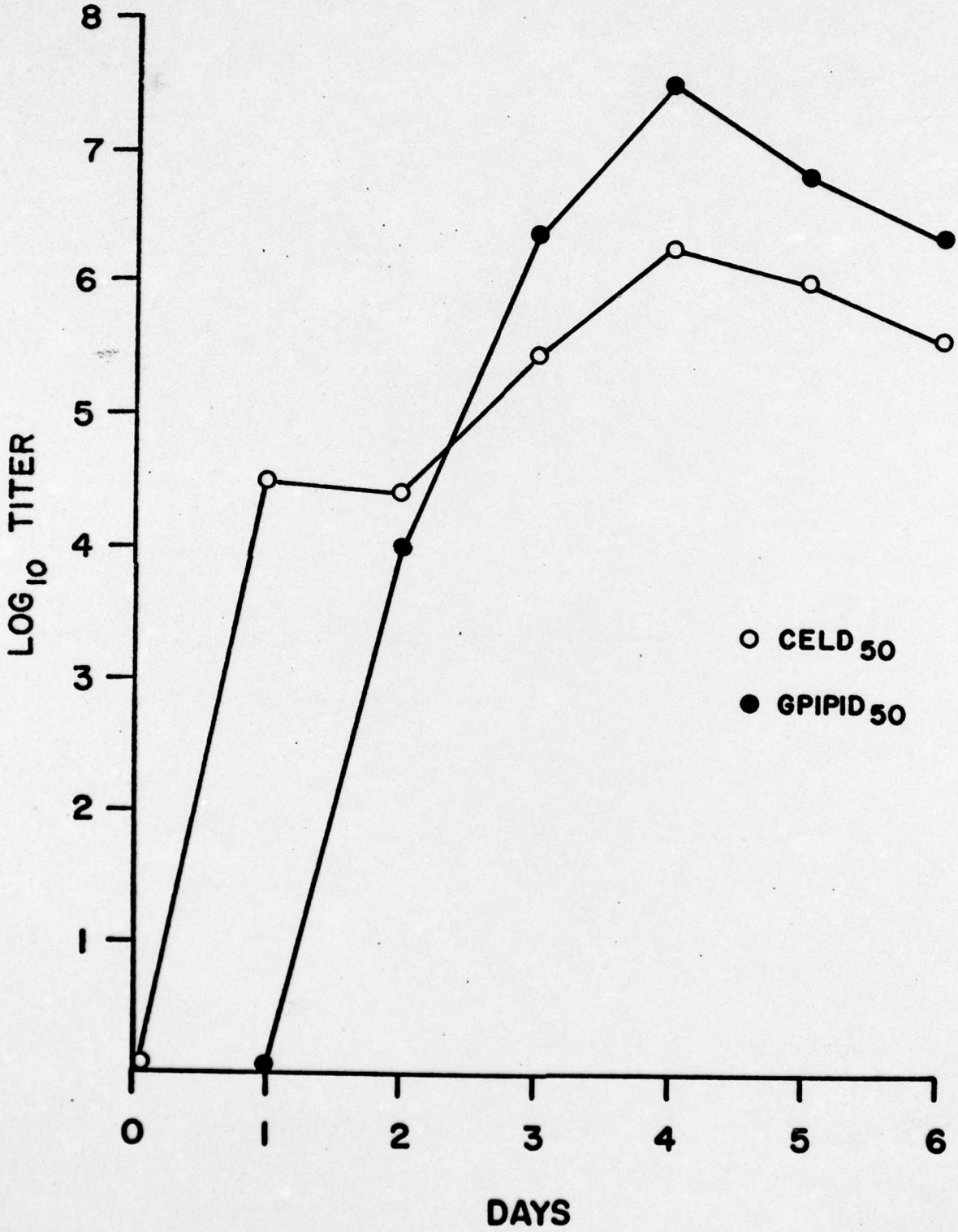
9. Wolf, G. L., C. R. Cole, H. N. Carlisle, and S. Saslaw. 1967. The pathogenesis of Rocky Mountain spotted fever in monkeys infected by inhalation. Arch. Pathol. 84:486-494.

TABLE 1. Dose response of R. rickettsii grown in DEC cultures and inoculated i.v. in rhesus monkeys (n=4)

Inoculum Log_{10}		Days (mean)		
$GPIPID_{50}$	$CELD_{50}$	Incubation period	Time to death	Duration of illness in survivors
4.0	4.4-4.5	2,2,4,4 (3.0)	6,_,_,_	_,8,5,8
6.3	5.4-5.6	1,1,2,1 (1.2)	3,5,_,_	_,_,7,10
6.8-7.5	6.0-6.2	1,1,1,1 (1.0)	3,4,4,5 (4.0)	--

LEGEND

FIG. 1. R. rickettsii titers by day postinfection in DEC cultures assayed by two methods: $CELD_{50}$ and $GPIPID_{50}$.


8
7
6
5
4
3
2
1
LOG10 TITER
0
1
2
3
4
5
6
DAYS
CELD50
GPIPID50

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle)
(6) Effect of Harvest Time of Rickettsia rickettsii from Duck Embryo Cell Cultures on Rocky Mountain Spotted Fever Infection in the Rhesus Monkey

5. TYPE OF REPORT & PERIOD COVERED
(9) Interim rept.

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s)
(10) Gerald L. Ruch [redacted] Duane E. Hilmas

8. CONTRACT OR GRANT NUMBER(s)

9. PERFORMING ORGANIZATION NAME AND ADDRESS
U.S. Army Medical Research Institute of Infectious Diseases SGRD-UIA-A
Fort Detrick, Frederick, Maryland 21701

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
(16) (17)
62776A | 3M762776A841 | 00 | 008

11. CONTROLLING OFFICE NAME AND ADDRESS
(11)

12. REPORT DATE
15 December 1976

13. NUMBER OF PAGES
10 (12) 12 pp

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)

15. SECURITY CLASS. (of this report)
UNCLASSIFIED

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

Reprints bearing assigned AD number will be forwarded upon receipt.
To be submitted for publication as a note in Applied and Environmental Microbiology.

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Rocky Mountain spotted fever
Rhesus monkey
Duck embryo cell culture
Infectivity

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This study was undertaken to determine the optimum harvest time for infected duck embryo cell (DEC) cultures to yield the highest rickettsial titers and greatest infectivity for rhesus monkeys. A consistently reproducible Rocky Mountain spotted fever-infected monkey model for pathogenesis studies and testing vaccine is produced when the inoculum given intravenously contains ≥ 10^6.0 chick embryo median lethal dose organisms. This is optimally attained with day-4 DEC culture harvest material.

1000000

405 039

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)